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Use of Activated Charcoal for Hemoperfusion in Dutch Rabbits

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SUMMARY

Techniques for a hemoperfusion system with activated charcoal have been developed for studying circulatory detoxification in Dutch rabbits. During a period of 8 hours of hemoperfusion, mean arterial blood pressure, heart rate, hematocrit, RBC fragility, plasma protein concentration, plasma osmolality, leukocyte and platelet counts, and rectal temperature did not show significant changes as compared with prehemoperfusion (base-line) values. All 5 tested rabbits survived after prolonged hemoperfusion, indicating that the present system is safe for use in rabbits.

Overdoses of drugs and intoxications with chemicals or toxins are important medical problems. Although standard procedures of supportive management have been established,[1] positive manipulative methods often are needed for removing circulating toxic substances. Hemoperfusion through activated charcoal for treatment of drug intoxication and poisoning recently has been reported for persons[2] and animals.[3,4,a] A problem encountered in the use of the hemoperfusion technique is the choice of a suitable adsorbent. Rosenbaum et al[5] showed that resin hemoperfusion was effective in reversal of drug intoxication, as well as the more commonly used coated or uncoated charcoal adsorbent. Gundermann and Lie[6] indicated that coated charcoal was safer than uncoated, but that the latter was considered to be more effective.[3] The objective of the present study was to develop a hemoperfusion system with activated charcoal as an adsorbent, using the Dutch rabbit as an animal model, in lieu of the subhuman primate (which is in short supply).[7]

Materials and Methods

Surgical Preparation of Rabbits—Cannulations were done of the external jugular vein and common carotid artery of 5 male Dutch rabbits (2.0 to 3.0 kg each) under Innovar-Vet[b] anesthesia (0.15 mg/kg, IM). Both polyethylene cannulae (ID 1.19 mm, OD 1.70 mm) were passed under the neck skin and out between the scapulas. The cannulae were filled with heparin (1,000 U/ml); the rabbit was placed in a restraint box.[c] Cannulae were then passed through a slit in the restraint box cover. The animals were given food and water ad libitum.

Assembly and Sterilization of Activated Charcoal Column—Activated charcoal and filters were obtained commercially.[d] The charcoal particles were sieved[e] to remove particles < 1 mm in size. The hemoperfusion column (with a capacity of 4 ml of blood and 5 g of charcoal) was constructed of plexiglass (Fig 1). The column, filled with activated charcoal, was flushed with triple-distilled water for 1 hour to remove any remaining small charcoal particles. Two sizes of Tygon tubing[f] (ID 0.97 mm, OD 0.40 mm; ID 4.75 mm, OD 19.05 mm), metal adapters, and Y and T tubes were connected to the column to trap air bubbles before the blood entered the venous cannula. The entire column was sterilized with ethylene oxide for 2 hours followed by air ventilation for 2 hours.

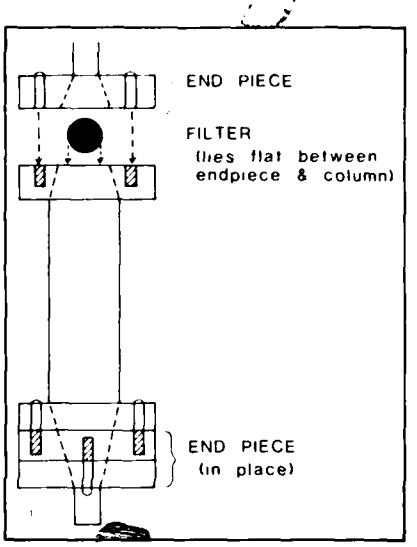

Fig 1—Assembly of a plexiglass hemoperfusion column.

Hemoperfusion Procedures—After a control blood sample (1.4 to 2.0 ml) was taken, 2 ml of heparin (1,000 U/ml) was injected IV. Fifteen minutes after the heparin injection, hemoperfusion was begun by starting the peristaltic pump[g] (Fig 2). Before opening the stopcock of the venous cannula for blood return to the rabbit, all air bubbles were removed from the system. Hemoperfusion was continued for 8 hours. During this period, a constant infusion of heparin (1,000 U/ml) was maintained at 1.16 ml/hour. The arterial blood flow through the column was maintained at 3.0 ml/hour. The volume of the entire hemoperfusion system was 8 ml.

Blood samples were obtained at 0 time (base line), at 10 minutes, and at 4-, 8-, and 24-hour intervals for analyses of hematocrit, plasma protein, total and differential WBC counts, erythrocyte fragility,[h] and plasma osmolality.[h] A maximum of 10

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[a] Liu CT, Sanders RP. Development of a hemoperfusion system with activated charcoal for rhesus monkeys (abstr). *Fed Proc* 39: 538, 1979

[b] Innovar-Vet, Pitman-Moore Inc, Washington Crossing, NJ.

[c] Plas Laboratories, Lansing, Mich.

[d] Hemocol, Warner-Chilcott, Morris Plains, NJ.

[e] USA standard testing sieve, 16 mesh, No. 18, Fisher Scientific Co, Pittsburgh, Pa.

[f] Silastic, Dow-Corning Corp, Midland, Mich.

[g] Buchler Instruments, Fort Lee, NJ.

[h] Automatic osmometer (Model A), Precision Systems, Waltham, Mass.

TABLE 1—Effects of Hemoperfusion in Dutch Rabbits (n = 5) with Activated and Coated Charcoal

Time (hours)	Mean arterial blood pressure (mm of Hg)	Heart rate (beats/minute)	Hematocrit (%)	Plasma protein (g/dl)	Count (No./mm³ × 10³) WBC	Count (No./mm³ × 10³) Platelet	Plasma osmolality (mOsm)	Rectal temperature (C)
0	103 ± 4.8	279 ± 21	31.4 ± 1.9	5.4 ± 1.1	10,445 ± 1,485	180 ± 34	287 ± 6.7	39.2 ± 0.2
10 minutes	99 ± 6.8	308 ± 17	33.9 ± 0.7	5.5 ± 1.1	11,988 ± 2,119	210 ± 64	289 ± 6.1	39.1 ± 0.3
4	106 ± 9.7	258 ± 19	31.2 ± 1.1	5.4 ± 1.4	9,768 ± 2,337	217 ± 107	296 ± 5.2	38.6 ± 0.4
8	91 ± 9.7	258 ± 9	31.6 ± 0.7	5.6 ± 1.1	16,708 ± 3,055	132 ± 27	293 ± 3.9	38.8 ± 0.6
24	100 ± 18.4	293 ± 26	29.8 ± 1.7	5.8 ± 1.1	17,080 ± 3,245	126 ± 38	290 ± 5.3	39.3 ± 0.5

Data are expressed as means ± SEM.

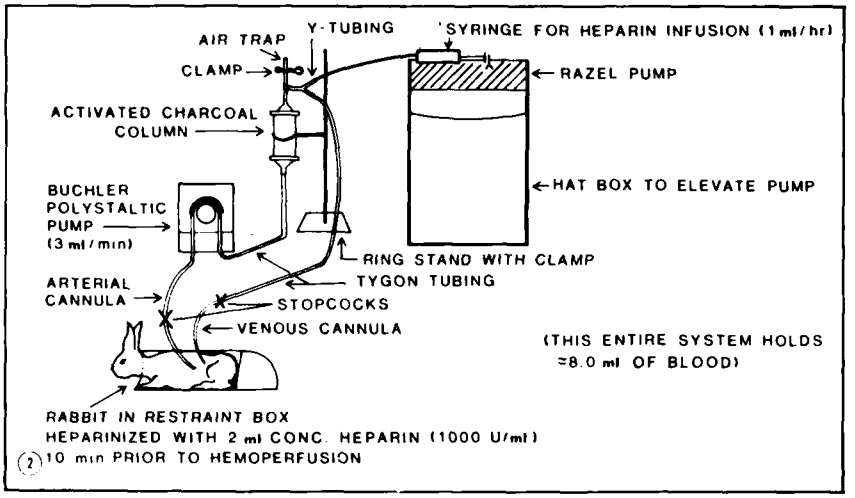

Fig 2—The entire activated charcoal hemoperfusion system connected to a Dutch rabbit.

ml of blood was taken from the common carotid artery during an 8-hour period of hemoperfusion. At the time of blood sampling, blood pressure and heart rate were determined with a pressure transducer[i] and recorder.[j] Rectal temperature[k] was recorded each hour.

Results and Discussion

This report appears to be the first description of the techniques for hemoperfusion through an activated charcoal column in conscious, restrained Dutch rabbits. Using this system, hemoperfusion caused no significant changes in any of the measured biochemical, hematologic, or cardiovascular values (Table 1). However, transient changes in heart rate, WBC counts, and erythrocyte fragility were seen in 3 of 5 rabbits. All variables returned to base line within 8 hours after the initiation of hemoperfusion. Although significant differences were not demonstrated between the baseline values and the subsequent values at 10 minutes and 4, 8, and 24 hours, some rabbits (2/5) showed respiratory difficulties (nasal congestion, discharge of nasal fluid, labored breathing) during hemoperfusion. The possible causes for respiratory changes might be due to local allergic reaction. However, 24 hours after hemoperfusion, all rabbits appeared normal.

Thrombocytopenia and hypotension after hemoperfusion with either coated[1] or uncoated[4] activated charcoal or with resin have been reported in persons. In the present study, these physiologic sequelae did not occur in the newly developed hemoperfusion system, using a coated, activated charcoal adsorbent in Dutch rabbits. Nevertheless, certain precautions were followed when using the system. Although formation of blood clots within the hemoperfusion system is often a problem, infusion of approximately 9,000 U of heparin over 8 hours prevented clotting and induced no adverse effects. Flow rate, an important factor in maintaining the safety of the system, was 3 to 5 ml/minute, which was found satisfactory for use in rabbits. A faster flow rate would increase the possibility of hemolysis or would harm the animal, and a slower rate would be less effective in removing chemical substances from the blood. Another precaution was to prevent excessive blood loss from blood sampling and the volume for extracorporeal circulation. The present hemoperfusion system was designed to contain approximately 8 ml of blood in the entire dead space, representing 5% to 6% of total blood volume of a Dutch rabbit.

Limitations for adsorbing low or middle molecular weight substances, using hemoperfusion systems, have been shown by Gundermann and Lie.[6] The present system has shown no effect in removing a high molecular weight protein substance (cholera enterotoxin[l]) in Dutch rabbits (unpublished observations). Hemoperfusion will be useful to remove substances from the blood that remain in the circulation for a reasonable period. If the substance leaves the circulation too quickly, if the half-life of a toxic substance is short, or if the substance cannot be adsorbed, the effectiveness of hemoperfusion will be minimal or negated.

[i] P23Db, Statham Instruments, Hato Rey, Puerto Rico.

[j] Brush Mark 240 Model, Gould Inc, Cleveland, Ohio.

[k] Model 43TA telethermometer and rectal probe, Yellow Springs Instrument Co, Yellow Springs, Ohio.

[l] Cholera enterotoxin, Schwartz-Mann, Orangeburg, NY.

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